3053-Pos
Single-Molecule Tracking of Nanorobots on Pseudo-One-Dimensional DNA Origami Tracks
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A synthetic molecular nanorobot dubbed a "spider" composed of a streptavidin protein "body" bound to three biotinylated 8-17 deoxyribozyme-bearing "legs" completes the programmed task of traversing a predetermined DNA origami track. The track is assembled by folding and holding in place a long single-stranded DNA molecule into a two-dimensional landscape by using hundreds of short oligonucleotides ("staples") and hybridizing chimeric DNA-RNA substrates to specific staples to realize a pseudo-one-dimensional track. Spiders are designed to undergo a biased random walk as their legs repeatedly cleave oligonucleotide substrates on a track, causing the legs to more readily dissociate from the cleaved product and progress towards the uncleaved substrate. We image Cy3-labeled spiders and Cy5-labeled origami using total internal reflection fluorescence microscopy (TIRFM). By fitting Gaussian functions to the imaged point spread functions, we are able to monitor with nanometer spatial precision the real-time motion of the spiders along DNA origami tracks.

3054-Pos
Direct Observation and Analysis of 3d Diffusing Fluorescent Proteins in Solution using Single Image Measurements
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The low temporal resolution of current single-molecule tracking methods on the order of 100 ms limits the methods' capability to investigate fast diffusion processes, such as a protein's 3D diffusion in solution. Using TIRF microscopy at sub-millisecond exposure times, we have directly imaged 3D-diffusing streptavidin-Alexa533 molecules in solution and recorded the intensity profiles of the diffusion proteins. The intensity profile of a 3D-diffusion protein represents a convolution of the native point spread function (PSF) of the molecule with the protein's pathway distribution function for the given exposure time, and can be approximated by a two-dimensional Gaussian function. The standard deviation (SD) of the Gaussian intensity profile of the 3D diffusing proteins allows us to obtain the molecule's diffusion coefficient to known precision [1], all by using a single image of sub-millisecond exposure time and our theoretical formulation relating SD of the intensity profile of a 3D-diffusing protein to the diffusion coefficient. Our theoretical formulation agrees with results using simulations and experimental studies on proteins of a known diffusion coefficient.
[1]. DeSantis, M. C., DeCenzo, S. H., Li, J. -L. & Wang, Y.M. Physical Review E. In Review.

3055-Pos
Towards Sub-Nanometer Resolution Optical Tweezers
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Optical tweezers with sub-nanometer resolution can provide new insights into how nucleic-acid associated molecular motors work, since elementary steps of one base-pair or less can be resolved. For example, elementary 0.34 nm steps of RNA-Polymerase, as well as of a viral packaging motor, have been observed with high-resolution optical tweezers instruments. Techniques for achieving ultra-stable trapping/microscopy include housing the instrument in a Helium atmosphere, temperature control, and stabilizing laser-pointing and intensity. We present results on optimizing the spatial resolution of our optical tweezers instrument, including experiments with a Helium atmosphere, fiber-coupled actively stabilized detection lasers, and precise temperature control of the trapping objective.

3056-Pos
Real-Time Super-Resolution Tracking of Single Deoxyribozyme Based Molecular Robots
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The probing and characterization of the behavior of individual nucleic acid based molecular robots is presented, using real-time single-particle tracking with super-resolution total internal reflection fluorescence microscopy (TIRFM). Nucleic acid based molecular assemblies, called "spiders", implemented as robots with multiple deoxyribozyme sensor-actuator legs traverse and cleave two-dimensional landscapes of surface bound oligonucleotide substrates. We analyze the movement of spiders to test the hypothesis that they walk by biased diffusion. The experimental approach demonstrated here should allow for control over the cybernetic properties of spiders, resulting in the integration and synthesis of complex robotic behaviors at the nanoscale based on DNA and RNA nanotechnology.

3057-Pos
Single-Molecule Dynamics of Conformational Interchange in Calmodulin
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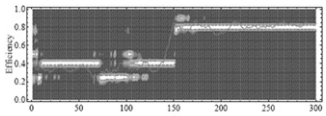
The recognition and binding of target binding domains by calmodulin (CaM) require global protein conformational changes. We describe single-molecule measurements of conformational interchange in CaM. CaM labeled with a FRET pair (Alexa Fluor 488 and Texas Red) was trapped in lipid vesicles tethered to the surface of a cover slip. Conformational transitions were observed on the time scale of 2 to 11 ms. The probability of a transition to a compact conformation was significantly lower at low than at high Ca^{2+} concentration, revealing more frequent transitions to a compact conformational state for CaM with bound Ca^{2+}. These results show that CaM undergoes functional conformational dynamics even in the absence of target enzyme. Conformational searching may permit CaM to readily adopt a binding geometry upon encountering a target binding domain.
FRET trajectories were also analyzed by a novel iterative Bayesian propagator to determine the probability distribution of FRET efficiencies as a function of time. We show that this analysis outperforms conventional running averages to resolve conformational jumps and conformational distributions. The figure shows a CaM FRET efficiency probability distribution (color scale). The red line shows the running average. The time axis is in milliseconds.



3058-Pos
Characterization of Single Molecule Protein Induced Fluorescence Enhancement (PIFE) as an Alternative to SmFRET
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Förster resonance energy transfer (FRET) is a widely used technique for real time single molecule detection. FRET measurement is based on distance changes between donor and acceptor fluorophores, hence the requirement for fluorophore attachment of the molecules under study. Single fluorophore labeling of protein is often difficult to achieve, rendering poor efficiency and specificity of labeling. Furthermore, proteins with high Kd which requires addition of high protein concentration would make single molecule detection impossible even with properly labeled proteins. Recently, PIFE, an alternative fluorescence assay was developed for probing translocational movement of an antiviral protein, RIG-I (1). PIFE employs a single fluorophore which exhibits enhanced quantum yield when approached by a protein at a close proximity. Although this photophysical effect is correlated with the lifetime change of the corresponding fluorophore (2) the sensitivity and the distance range of the method needs to be further characterized.
We performed a systematic study of a single molecule PIFE where we monitored binding of a restriction enzyme BamHI to Cy3 labeled DNA. Preliminary data shows that the sequence specific binding of BamHI to DNA in a buffer containing calcium results in an increase of Cy3 intensity. The overall level of fluorescence increase shows dependence on the protein concentration and single molecule traces display binding and unbinding as discrete steps of increase and decrease in Cy3 intensity respectively. We confirmed the sequence specific cleavage activity of BamHI triggered by the presence of magnesium.
References
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2. Sorokina M. et al., ''Fluoresecent Lifetime Trajectories of a Single Fluorophore Reveal Reaction Intermediates During Transcription Initiation'', J Am Chem Soc 131, 9630-9631 (2009).